PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

COPY

To:

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.03.2001
Applicant's or agent's file reference SP/GM/N9557	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00740	01/03/2000	02/03/1999

Applicant
KING'S COLLEGE LONDON

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

COPY

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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SP/GM/N9557		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00740	01/03/2000	02/03/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant KING'S COLLEGE LONDON		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/09/2000	29.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hoesel, H Telephone No. +49 89 2399 8693

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00740

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-3,5-20	as originally filed			
4,4a	as received on	05/03/2001	with letter of	27/02/2001

Claims, No.:

1-38	as received on	05/03/2001	with letter of	27/02/2001

Drawings, sheets:

1/1	as originally filed

Sequence listing part of the description, pages:

1 - 13, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00740

listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 16, 20 (all entirely); 17 - 19, 21 - 23, 27 (all partially).

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 16, 20 (bothe entirely); 17 - 19, 21 - 23, 27 (all partially).

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00740

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1 - 19, 21 - 38.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 15, 17 - 19, 21 - 38
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1 - 15, 17 - 19, 21 - 26, 29, 30, 32, 33, 35 - 38
	No:	Claims	27, 28, 31, 34
Industrial applicability (IA)	Yes:	Claims	1 - 38
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00740

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB00/00740

Reference is made to the following documents:

D1: WO 88 03957 A
D2: WO 90 14444 A
D3: US-A-5,582,978
D4: US-A-5,521,300
D5: WO 96 24686 A
D6: WO 96 00298 A
D7: LEW & DESMARCHELIER, J. CLIN. MICROBIOL. vol. 32/5, 1994, p 1326-1332
D8: US-A-5,292,874

SECTION III:

1. As the applicant had paid only 9 of 25 additional search fees requested by the international search authority, the search was limited to the subject-matter and the sequences encompassed by claims 3 and 5 (as requested in the applicant's letter of 16.08.2000).

Thus examination has been carried out for the following oligonucleotides only:
Seq Id Nos: 3, 4 (specific for *Proteus mirabilis)*
Seq Id Nos: 5, 8, 10, 37, 48 (specific for *E. coli)*
Seq Id Nos 6 and 7 (specific for *Klebsiella* species)
Seq Id No: 9, 38, 49 (specific for *Enterobacter cloacae*)
Seq Id Nos: 20 - 26 (specific for *Staphylococcus* species)
Seq Id Nos: 15, 18 (specific for *Streptococcus pneumoniae* "Pneumococci")
Seq Id No: 13 (specific for *Pseudomonas aeruginosa)*
Seq Id Nos: 16 and 19 (discriminating between *Enterococcus* species)
Seq Id No: 28 (specific for *Strenotrophomonas maltophilia*)
Seq Id No: 27 (specific for *Burkholderi cepacia)*
and additionally the sequence identified as Seq Id No 29 (*Listeria* sp) which has been searched for.

No examination is carried out for subject-matter for which no search has been done. This applies to claims 17 - 19, 21 - 23 and 27, insofar as these extend to

unsearched nucleic acid sequences and combinations thereof, and to claim 20.

Search for the subject-matter of independent claims 1, 13 and 25 has been carried to such extent that an assessment of novelty and inventive steps for these and their dependent claims can be carried out.

2. Claim 16 which has been filed after the search report has been completed represents an extension of the subject-matter of claims 17 - 20 as originally filed to include oligonucleotides different from those actually disclosed. Consequently, no search has been performed over the extended scope of the claim.

In the absence of an extensive search, an assessment of novelty and inventive step of the subject-matter covered by claim 16 cannot be carried out.

SECTION IV:

3. The international examining authority considers the application to lack unity (Rule 13.1 PCT) with respect to the product claims 27 - 38 for the following reasons:

3.1. These claims are directed to isolated oligonucleotides of defined sequences.

According to the applicant's opinion the common concept linking together these claims with independent claims 1, 13 and 25 is established by the fact that the probes are designed to hybridize to a particular amplicon. It is noted that this argument would apply only if the said claims accordingly reflect a said use of the probes in an amplification protocol using a selected set of primers. However, contrary to a claim directed to the use of an oligonucleotide in a method..., this is not the case with claims which relate to the oligonucleotide as such.

Consequently, the common concept of the product claims must be formulated as to provide further probes that hybridize to a selected region within the 23S rRNA that corresponds to nucleotides **107 - 478** of the *E.coli* 23S rRNA.

3.2. It is repeated that the different oligonucleotides covered by claims 27 - 38 neither share a technical relation in terms of a homologous sequence motif nor in terms of

a particular specificity towards one selected taxonomic group of bacteria. Contrary to a use claim, the present product claims do not imply any limitation as to a particular use (such as in a method of claim 1). In contrast thereto, the claimed probes can be used for other hybridization methods with or without preceding PCR or alternatively could by used as (more or less) species specific primers.

In addition, the underlying technical problem to detect a variety of bacteria in PCR based method using a single pair of primers (as addressed by the method of claim 1), is not solved by the isolated oligonucleotide, but requires the provision of sets or combinations thereof wherein each of the probes is selected for having substantially the same hybridization strength and selectivity.

Thus, it is maintained that the products of claims 27 - 38 cannot be considered as "specifically adapted" for carrying out the present method (cf. the PCT-Guidelines, Ch-III. 7.3).

3.3. Inventiveness of the common concept:
It is known in the state of the art that this region of the 23S rRNA varies within the different taxonomic groups of bacteria and thus may serve as the source of taxon specific probes or primers of clinical interest.

(i) D1, for instance, discloses the following probes derived from the present region of interest (the base numbering refers to the *E.coli* rRNA system):

Probes specific for the genus *Legionella:* **350 - 395** (claim 117)
Probes specific for *Chlamydia trachomatis*: **275 - 320** and **330 - 365** (cl. 145 and 147)
Probes specific for *Enterobacter cloacae*: **305 - 430** (cl. 192)
Probes specific for *Proteus mirabilis*: **270 - 305** (claims 195 and 199, cf. Seq Id No 4)
Probes specific for the genus *Salmonella*: **335 - 375** (cl. 209).
Probes specific for highly conserved regions: **460 - 490** (cl. 235):

(ii) Other probes derived from the said region of 23S rRNA are probe 1336

(corresponding to nucleotides **274 - 301** specific for *S. aureus* and some strains of *S. epidermidis*) and probe 1337 (**304 - 335**, specific for *S. aureus*, see D2, Tables 1 and 2).

(iii) D3 discloses probe 1984 which is specific for *H. influenzae* and which corresponds to nucleotides **343 - 356** of E.coli 23S rRNA (Figure 2, table 1 and col. 13, line 51 - 54).

(iv) D4 discloses probes specific for the genus *Mycobacteria* or for particular mycobacterial species, as well as their use in hybridisation-based and PCR-based identification methods, particularly probe p 2364 (specific for *M. tuberculosis* hybridizing to a region corresponding to *E.coli* 23S rRNA **254 - 284**); the probes p2382 (*M. kansasii*) and p.2373 (*M. fortuitum*) have the same localization(cf. col. 1, lines 36 - 40 and line 64 - col. 2, line 6, col. 11, lines 27 - 41, table 2, lines 47 - 62, tables 3b, 3c, 3e).

(v) D8 discloses a probe that is capable of distinguishing *S. aureus* from other staphylococci. This probe hybridizes to a region corresponding to bases 338 - 367. Furthermore two helper probes that hybridize to regions adjacent to the target region of the discriminatory probe (col. 2, line 55 - col. 3, line 4, col. 8, lines 35 - 46) are disclosed.

3.4. Thus, the relative variability of the target region of interest of the 23SrRNA has been recognized and exploited in the prior art by the provision or the "design" of various taxon specific probes. According to the applicant's arguments, each of the oligonucleotides referred to above should be considered as specifically designed for the present method.

Consequently, each of the above-mentioned documents anticipates the common concept linking together the various alternative oligonucleotide sequences covered by claims 27 - 38.

4. The non-unity objection does not extend to claim directed to sets of oligo-nucleotides (Claims 16 - 24). This set of probes can be considered as specifically designed for the method of the present invention by the fact that each of the

probes of the set must be selected to hybridize within the region of interest. The same would apply to a claim directed to a kit comprising **the primer pair** as specified in claim 1 **in combination** with at least one of the probes listed in claims 17 to 19 or **in combination** with a solid support material as specified in claim 27. These types of test kits that contain **all essential reaction components** are considered to be specially adapted for carrying out the method of claim 1.

5. Thus, the application contains the following separate inventions:

(1): method of identifying bacteria by means of amplification using a selected pair of primers, followed by hybridization with a set of probes, accordingly adapted set of primers, sets of probes, test kits (claims 1 - 26)
(2) probes specific for *Proteus mirabilis* (claim 27 in part, claim 28)
(3) probes specific for *E. coli* (claim 27 in part, claim 29)
(4) probes specific for *Klebsiella* species (claim 27 in part, claim 30)
(5) probes specific for *Enterobacter cloacae* (claim 27 in part, claim 31)
(6) probes specific for *P. aeruginosa* (claim 27 in part, claim 32)
(7) probes discriminating between *Enterococcus* species (claim 27 in part, claim 33)
(8) probes specific for *Staphylococcus* species (claim 27 in part, claim 34)
(9) probe specific for *Burkholderi cepacia* (claim 27 in part, claim 35)
(10) probe specific for *Strenotrophomonas maltophilia* (claim 27 in part, claim 36)
(11) probe specific for *Listeria* sp. (claim 27 in part, claim 37)
(12) probes specific for Pneumococci (claim 27 in part, claim 38)

SECTION V:

6. Invention 1:

6.1. The particular combination of technical features, i.e. the selection of the pair of primers, which combination warrants for amplification of bacterial 23S rRNA of many different taxonomic groups of bacteria, is neither disclosed nor derivable in an obvious manner from the above-mentioned prior art, when taken into consideration either alone or in combination.

Thus, the method and the products according to claims 1 - 15 and 25 are novel and inventive in the sense of Art. 33(3) and (3) PCT.

6.2. Although single probes that are capable of hybridizing to the amplicon of the present invention are known, the prior art neither discloses nor suggests their combination into a set. The additional feature of claim 24 (in order to increase the hybridization signal of intensity of weakly reactive probes) is neither taught nor suggested by the prior art taken into consideration.

Consequently, the subject-matter of claims 17 - 19, 21 - 24 (to the extent they have been searched) is considered to be novel and inventive, as required by Art. 33(2) and (3) PCT.

7. Inventions 2 - 12:

7.1. Oligonucleotides having sequences identical with those given in Seq Id Nos. 3 - 10, 13, 15, 16, 18 - 29, 37, 38, 48, 49 are not disclosed in the prior art. The said subject-matter can therefore be regarded as novel in the sense of Art. 33(2) EPC.

7.2. (i) D1 discloses oligonucleotides derived from *Proteus mirabilis* 23 S rRNA 270 - 305 (claim 199) are specific for this organism. A particularly preferred one is that disclosed in claim 195 and Seq Id No 4. This oligonucleotide overlaps with the complement of Seq Id No 3 in 24 contiguous nucleotides. A skilled person would regard the preparation and use of other oligonucleotides of the indicated stretch, such as that given in Seq Id No 3 as obvious equivalents of the probe of claim 195.

Consequently, the subject-matter of claims 27 and 28, insofar as Seq Id No 3 is concerned, lacks an inventive step, contrary to Art. 33(3) PCT.

7.3. D1 furthermore indicates that probes specific for *Enterobacter cloacae* can be derived from 305 - 430 (cl. 192). The sequence given on p 90, line 22 shows identity in 18 nucleotides within a 20 base overlap with that of Seq Id No 9. A skilled person would be motivated by this disclosure to design similar, eventually improved probes from the said region of *Enterobacter cloacae* rRNA.

Consequently, D1 renders obvious the subject-matter of claims 27 and 31, insofar as Seq Id No 9 is concerned (Art. 33(3) PCT).

7.4. It is noted that the complement of the helper probe Seq Id No 2 of D8 has sequence identity in 21 bases in a 22 base overlap with the probe of Seq Id No. 21. The helper probe appears to be specific for the target organism in order to increase the reliability of the hybridization.

In view of the disclosures of D8, a skilled person would regard the provision of further equivalent probes derived of the region of interest as an obvious option. Thus, D8 appears to render obvious the subject-matter of claims 27 and 34 insofar as Seq Id No 21 is concerned (Art. 33(3) PCT).

7.5 The prior art referred to does not disclose discriminatory probes having sequences overlapping with Seq Id Nos 4 - 8, 10, 13, 15, 16, 19, 20, 22 - 29, 37, 38, 48 and 49.

Thus, the subject-matter of claims 29, 30, 32, 33, 35 - 38 is considered to be inventive in the sense of Art. 33(3) PCT.

SECTION VII:

8. Table 1 is inconsistent in its sequence numbering with the list given on p. 5 - 10 (a sequence having the numbering 27 does not exist, for instance).

The PCR products produced by these primers, from a range of medically important Gram positive and Gram negative bacterial cultures, are characterized by hybridization to an array of oligonucleotides designed to identify taxonomic groups. Using this procedure, which takes typically less than four hours, we have been able to identify a wide range of genera and species. This approach allows bacteria and mixtures of bacteria to be identified by molecular methods without the need for a priori knowledge of the causative agent or agents.

In summary, the present invention comprises a method for identifying bacteria in a test sample which comprises amplifying a portion of the 23S rDNA present in the sample using a primer pair comprising one primer consisting essentially of one or more oligonucleotides having the sequence or sequences

5'GCGATTTCYGAAYGGGGRAACCC

and a second primer consisting essentially of an oligonucleotide having the sequence

5'TTCGCCTTTCCCTCACGGTACT.

and testing the resulting amplicon by probing a set of oligonucleotides designed to identify bacteria which may be present in the sample by hybridising to their respective amplicon. In a set of oligonucleotides suitable for use with this method, the oligonucleotides are designed to hybridise to the products of the amplification reaction in a single test and therefore under a single set of hybridisation conditions.

International application WO 88/09397 describes the preparation of numerous oligonucleotide probes which hybridise to certain regions of 16S and 23 S ribosomal nucleic acid. International application WO 90/14444 and US patents 5,592,978, 5,521,300 and 5,292,874 describe the preparation of individual probes which bind to certain regions of ribosomal nucleic acid but which are specific for one species of organism or one genus or sub-generic classes thereof. However, in contrast to the present invention, none of these publications disclose, either in concept or in reality, sets of oligonucleotides designed to work in unison by hybridising to a uniquely specified region of 23S ribosomal nucleic acid after amplification of bacterial nucleic acid with one specific pair of amplification primers. According to the present

invention, the sets of oligonucleotides which may be used hybridise in parallel to a range of amplicons under the same hybridisation conditions and can therefore be used in a single test for the identification of a range of different organisms.

Oligonucleotide probes, the sequences of which are set out below, have proved highly successful when used in various combinations in tests typically carried out in hospitals. They can be used in a panel or array for the identification of many different species. There is theoretically no limit to the number of oligonucleotide targets employed and the number of species that can be identified. Ideally the oligonucleotides used should each hybridize only to one bacterial species and to all members of that species. Thus with an ideal array, a unique profile consisting of species specific spots would be seen, giving identification to the species level. In practice, two or more oligonucleotide spots may be required for many species and in some cases several such spots may allow identification of variation within a species. In addition, some identifications can be made by comparing the relative intensities of hybridization of individual species to individual oligonucleotides. The assessment of hybridization can be quantified by visual or automated methods.

For example, 27 oligonucleotides have been used for the unambiguous identification of Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus feacium and Enterococcus feacalis , as well as Staphylococcus aureus, coagulase negative Staphylococcus, Listeria species, Stenotrophomonas maltophilia , Burkholderia cepacia, and Escherichia coli. Usually, therefore, it will be desirable to provide oligonucleotides to probe not only for the 8, 10, or more of the micro-organisms commonly occurring in hospital samples or the samples being tested in other situations, but also for other organisms likely to be encountered. Preferably, probes for at least 30 different species of micro-organism will be present on the support substrate used in the test.

CLAIMS

(1) A method for identifying bacteria in a test sample which comprises amplifying a portion of the 23S rDNA present in the sample using a primer pair comprising one primer consisting essentially of one or more oligonucleotides having the sequence or sequences

5'GCGATTTCYGAAYGGGGRAACCC

and a second primer consisting essentially of an oligonucleotide having the sequence

5'TTCGCCTTTCCCTCACGGTACT.

and testing the resulting amplicon by probing a set of oligonucleotides designed to identify bacteria which may be present in the sample by hybridising to their respective amplicon.

(2) Method according to claim 1, in which at least 8 bacterial species are tested for.

(3) Method according to claim 2, in which the organisms tested for comprise at least one of Escherichia coli , Staphylococcus aureus , Pseudomonas aeruginosa , Enterococcus spp. , Klebsiella spp. , Enterobacter spp. , Proteus spp, Pneumococci, and coagulase negative Staphylococci.

(4) Method according to claim 1, in which at least 10 bacterial species are tested for.

(5) Method according to claim 4, in which the organisms tested for comprise at least one of Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus feacium, Enterococcus feacalis , Staphylococcus aureus, coagulase negative Staphylococcus, Listeria species, Stenotrophomonas maltophilia , Burkholderia cepacia, and Escherichia coli.

(6) A method according to claim 1, in which the oligonucleotides have sequences selected from the group consisting of

SEQ ID Nos 3-7, 9-13, 15-19, 21-28, 30-32, 39-41, 44-49, 51, and 53-58.

(7) A method according to claim 1, in which the oligonucleotides have sequences selected from the group consisting of
SEQ ID Nos 8, 14, 20, 29, 33-38, 42, 43, 50, 52, and 59.

(8) A method according to claim 1, in which the oligonucleotides have sequences selected from the group consisting of
SEQ ID Nos 3-59.

(9) A method according to claim 1, in which the oligonucleotides have sequences selected from the group consisting of
SEQ ID Nos 60 -63.

(10) A method according to any of claims 1 to 9, in which amplification is carried out by the polymerase chain reaction (PCR)

(11) A method according to any of claims 1 to 9, in which amplification is carried out by transcription mediated amplification.

(12) A method according to any of the preceding claims, in which the set of oligonucleotides are attached to a support material.

(13) A primer pair comprising one primer consisting essentially of one or more oligonucleotides having the sequence or sequences
5'GCGATTTCYGAAYGGGGRAACCC
and a second primer consisting essentially of an oligonucleotide having the sequence
5'TTCGCCTTTCCCTCACGGTACT.

(14) A primer pair according to claim 13, of which one is a labelled primer.

(15) A primer pair according to claim 13, of which one is a digoxigenin-labelled primer.

(16) A set of oligonucleotides for identifying specific bacteria present in a test sample, comprising oligonucleotides designed simultaneously to identify different bacterial species which may be present, the oligonucleotides being capable of hybridising to a segment of bacterial 23S ribosomal nucleic acid amplified by the use of the primers specified in claim 13, 14, or 15.

(17) A set of Oligonucleotides having sequences selected from the group consisting of SEQ ID Nos 3-7, 9-13, 15-19, 21-28, 30-32, 39-41, 44-49, 51, and 53-58.

(18) A set of Oligonucleotides having sequences selected from the group consisting of SEQ ID Nos 8, 14, 20, 29, 33-38, 42, 43, 50, 52, and 59.

(19) A set of Oligonucleotides having sequences selected from the group consisting of SEQ ID Nos 3-59.

(20) A set of Oligonucleotides having sequences Selected from the group consisting of SEQ ID Nos 60 -63.

(21) A set of oligonucleotides according to any of claims 16 to 20, on a support substrate.

(22) A solid support material carrying a set of oligonucleotides as specified in any of claims 16 to 20.

(23) A support material according to claim 21 or 22, in which some or all of the oligonucleotides are attached to the support by means of chemically modified or additional bases.

(24) A support material according to claim 23, in which additional thymine bases have been attached to the 3 prime end of the oligonucleotide to increase hybridization intensity.

(25) A diagnostic kit for the identification of bacteria comprising an amplification primer pair according to claim 13, 14, or 15, and a set of oligonucleotides according to any of claims 16 to 21.

(26) A diagnostic kit according to claim 25, in which the oligonucleotides are on a support substrate.

(27) An oligonucleotide having a sequence which is any one of the sequences from SEQ ID No 3 to SEQ ID No 63 inclusive.

(28) An oligonucleotide for identifying *Proteus mirabilis*, having the sequence of SEQ ID No 3 or No 4.

(29) An oligonucleotide for identifying *Eschericia coli*, having the sequence of SEQ ID No 5, No 8, No 10, No 37, or No 48.

(30) An oligonucleotide for identifying a *Klebsiella* species, having the sequence of SEQ ID No 6 or No 7.

(31) An oligonucleotide for identifying an *Enterobacter* species, having the sequence of SEQ ID No 9, No 38 or No 49.

(32) An oligonucleotide for identifying *Pseudomonas aeruginosa*, having the sequence of SEQ ID No 13.

(33) An oligonucleotide for identifying *Enterococci*, having the sequence of SEQ ID No 16 or No 19.

(34) An oligonucleotide for identifying *Staphylococcus* species, having the sequence of any of SEQ ID Nos 20 to 26.

(35) An oligonucleotide for identifying a *Burkholderia* species, having the sequence of SEQ ID No 27.

(36) An oligonucleotide for identifying a *Stenotrophomonas* species,having the sequence of SEQ ID No 28.

(37) An oligonucleotide for identifying a *Listeria* species, having the sequence of SEQ ID No 29.

(38) An oligonucleotide for identifying *Streptococcus pneumoniae*, having the sequence of SEQ ID No 15 or No 18.